Remarks

Claims 42, 52, 56, 57, 59, 64-67, 69, 71-75, and 77-83 were pending in the subject application. By this Amendment, claims 42, 56, 72, and 78 have been amended and claims 59, 71, 77, and 82 have been cancelled. The undersigned avers that no new matter is introduced by this Amendment. Support for the amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 42, 52, 56, 57, 64-67, 69, 72-75, 78-81, and 83 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

Submitted herewith is a supplemental Information Disclosure Statement (IDS), accompanied by the form PTO/SB/08 and copies of the references listed therein. Applicants respectfully request that the references listed on the form PTO/SB/08 be considered and made of record in the subject application.

As an initial matter, Applicants gratefully acknowledge the Examiner's indication that claims 56 and 78 are objected to but would be allowable if rewritten into independent form to include the limitations of any base and intervening claims.

By this Amendment, claims 42 and 72 have been amended to recite that the siRNA molecule targets a sequence within the 3' non-coding region of the DV genome that is common to four serotypes of DV. Support for these amendments can be found, for example at page 3, lines 3-9, and page 32, lines 5-32 of the specification as filed. In addition, the paragraph at page 9, lines 14-17, within the Brief Description of the Sequences section of the specification has been amended to remove the phrase "of the prM gene". This is an obvious error in view of page 3, lines 3-9 of the specification; page 32, lines 5-16 of the specification; the sequence of GenBank Accession No. M29095; and the sequence of SEQ ID NO:4 (DEN-si-3'UTR) itself.

Claims 42, 52, 59, 64, 69, 71-73, 75, 77, and 80-82 are rejected under 35 USC §103(a) as obvious over Iversen *et al.* (U.S. Published Application 2005/0096291), Raviprakash *et al.* (*J Virol,* 1995, 69(1):69-74), Adelman *et al.* (*J Virol,* 2002, 76(24):12925-12933), Tuschl *et al.* (U.S. Patent 7,056,704), and Yu *et al.* (*PNAS*, 2002, 99(9):6047-6052). In addition, claims 57 and 83 are also

rejected under 35 USC §103(a) as obvious over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.* (*PNAS*, 2002), as applied to claims 42, 52, 59, 64, 69, 71-73, 75, 77, and 80-82 above, and further in view of Yu *et al.* (U.S. Patent 6,852,528). Furthermore, claims 65-67, 74, and 79 are rejected under 35 USC §103(a) as obvious over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.* (*PNAS*, 2002), as applied to claims 42, 52, 59, 64, 69, 71-73, 75, 77, and 80-82 above, and further in view of Hope *et al.* (U.S. Patent 6,136,597). Applicants respectfully traverse.

At page 6, the Office Action indicates that "it would have been obvious to target any of the DV genomic RNA regions set forth by Iversen *et al.*, Raviprakash *et al.*, or Adelman *et al.* because the genome of DV is a positive strand RNA encoding a single polyprotein, such that cleavage of any of the targets would have been expected to inhibit DV replication, particularly those at the 5' genome end, *e.g.*, those directed against prM." As indicated above, by this Amendment, claims 42 and 72 have been amended to recite that the siRNA molecule targets a sequence within the 3' non-coding region of the DV genome that is common to four serotypes of DV.

As indicated at page 3, lines 3-9 of the specification, and the Introduction section of the Zhang W. *et al.* publication (*Genetic Vaccines and Therapy*, 2004, 2(8):1-10), which is co-authored by the inventors and of record, the 3' non-coding region (NCR) is a 400 to 600 nucleotide-long region flanking the open reading frame of the polyprotein (C-prM-E-NS1-NS2a-NS3-NS4a-NS4b-NS5). This 3' NCR is predicted to form a stem-and-loop secondary structure (see page 32, lines 5-15, of the specification and the Discussion section of Zhang W *et al.*).

It is known in the art that the effectiveness of siRNA varies with the targeted position of the mRNA. This is supported by Shao *et al.*, "Effect of Target Secondary Structure on RNAi Efficiency", *RNA*, 2007, 13:1631-1640, which is of record, and the following publications, which are included with the supplemental IDS submitted herewith: Schubert *et al.*, "Local RNA Target Structure Influences siRNA Efficacy: Systematic Analysis of Intentionally Designed Binding Regions", *J. Mol. Biol.*, 2005, 348, 883–893; Schubert *et al.*, "Oligonucleotide-Based Antiviral Strategies," *Handb. Exp. Pharmacol.*, 2006, 173:261-287; Kreuger *et al.*, "Insights into Effective RNAi Gained from Large-Scale siRNA Validation Screening," *Oligonucleotides*, 2007, 17:237-250; Luo *et al.*, "The Gene-Silencing Efficiency of siRNA is Strongly Dependent on the Local Structure

of mRNA at the Targeted Region," *Biochemical and Biophysical Research Communications*, 2004, 318:303–310; Jayasena S., "Designer siRNAs to overcome the challenges from the RNAi pathway," *Journal of RNAi and Gene Silencing*, 2006, 2(1):109-117; and Rutz *et al.*, "Towards *In Vivo* Application of RNA Interference – New Toys, Old Problems," *Arthritis Research & Therapy*, 2004, 6(2):78-85). For example, Schubert *et al.* state "our findings indicate that, although a favourable siRNA sequence is a necessary prerequisite for efficient RNAi, complex target structures may limit the applicability even of carefully chosen siRNAs" (abstract), and "here, we report that gene silencing by a potent siRNA is diminished drastically when target nucleotides are incorporated into various hairpin structures" (page 884, first column). In particular, page 273, first full paragraph, of Schubert *et al.* (2006); page 111, first full paragraph of Jayasena S. (2006); and Table 1 at page 80 of Rutz *et al.* show that when screening potential mRNA target sites, regions likely to have complex secondary structure or to bind regulatory proteins are often eliminated in favor of other more accessible mRNA regions. The Tuschl *et al.* patent is cited in the Office Action for teaching the advantages of siRNAs. The Tuschl *et al.* patent itself teaches that a lack of reduction of RL luciferase expression in 293 cells may have been due to limited accessibility of the target sequence due to RNA secondary structure or associated proteins (column 22, lines 49-58, and column 28, lines 64-67). Thus, in view of the teachings and knowledge in the art at the time the application was filed, selection of the DV 3' NCR for targeting the siRNA molecular would have been counterintuitive to those of ordinary skill in the art, particularly in view of the large coding region for the DV polyprotein that was available for target selection. In addition, even if one of ordinary skill in the art were to proceed counter to the aforementioned teachings and select the DV 3' NCR for the target sequence, in view of its predicted secondary structure, the references cited in the rejections under 35 USC §103(a) do not provide those of ordinary skill in the art with a reasonable expectation that a sequence within the NCR that is common to four DV serotypes would be accessible and successfully targeted for RNA interference and result in inhibition of DV infection *in vivo*.

The Subramanya *et al.* publication (*Journal of Virology*, Mar. 2010, 84(5):2490-2501), of record, supports the non-obviousness of the claimed invention, and was published more than five years after the application's filing date. As described in Subramanya *et al.*, dendritic cells are of special relevance to dengue virus infection, and a hurdle for RNAi therapeutics is "the specific

delivery of small interfering RNA (siRNA) to relevant cell types" (page 2491, left column, first full sentence):

> "Dengue-infected DCs play a key role in the immunopathogenesis of DHF/DSS, as, along with macrophages, they release proinflammatory cytokines and soluble factors that mediate plasma leakage, thrombocytopenia, and hypovolemic shock associated with severe dengue infection (14, 15, 29, 38). Therefore, development of a method to introduce siRNA into DCs would be an important step toward using RNAi therapeutically to suppress viral replication and/or to attenuate the vigorous host cytokine responses in dengue infection (7, 19)" (page 2491, left column, first full paragraph, of Subramanya *et al.*, emphasis added).
>
> "DCs are of special relevance to dengue infection, as they are the initial cells in the skin to become infected during transmission of the virus by infected mosquito bite (12), and the proinflammatory cytokines that they produce play a significant role in dengue immunopathogenesis (13, 14, 38)" (page 2497, left column, last paragraph, of Subramanya *et al.*).

This important step of developing a method to introduce dengue virus-targeted siRNA into dendritic cells (DCs) to inhibit viral replication was first achieved by the inventors of the subject invention. Indeed, Subramanya *et al.* indicate that nonselective methods have been used successfully for *in vivo* delivery of siRNA to liver and other tissues; "however, they may not work well for primary hematopoietic cells such as DC" (page 2498, left column, of Subramanya *et al.*, emphasis added). As described in Examples 8 and 9 of the subject application, the inventors of the subject invention determined that interfering RNA targeting DV can effectively be delivered to human dendritic cells, decrease dengue virus infection, and inhibit dengue virus-induced apoptosis of these cells. In contrast, the primary reference relied upon in each of the rejections under 35 USC §103(a), the Iversen *et al.* publication, describes an experiment in which antisense oligonucleotides inhibited replication in Vero cells, which are kidney epithelial cells of the African Green Monkey (see Example 3, at page 15, paragraphs [0179] and [0180] of the Iversen *et al.* publication). Uptake of the antisense oligonucleotides by dendritic cells and inhibition of dengue virus infection of dendritic cells was not evaluated in the Iversen *et al.* publication.

At page 11, the Office Action states that "Applicant has presented no clear evidence that one of ordinary skill would have doubted that at least some DCs would have been transfected by the methods of the combined references, and that DV gene expression would have been inhibited in

those transfected cells" (emphasis added). The claims as amended recite more than transfection of some DCs. For example, independent claim 42 as amended recites a method for attenuating DV infection in human cells susceptible to DV infection *in vivo*, and administration of an effective amount of the vector. Claim 69 recites that the siRNA molecule attenuates DV replication in the cells. Independent claim 72 recites a method for inhibiting DV infection and DV-induced apoptosis of human dendritic cells *in vivo*, and administration of an effective amount of the vector. Claim 73 recites that the cells are subsequently exposed to DV, and the siRNA molecule inhibits DV infection and DV-induced apoptosis in the cells. In rejecting claims as *prima facie* obvious under 35 USC §103(a), it is the burden of the Patent Office to establish that the teachings of the prior art provide a sufficient basis for a reasonable expectation of success.

In the Raviprakash *et al.* publication, modified phosphorothioate antisense oligonucleotides and unmodified antisense oligonucleotides were injected into LLCMK/2 cells, which are cells of a rhesus monkey kidney cell line, not dendritic cells. Furthermore, the results of the Raviprakash *et al.* publication, independently or in combination with the other cited references, would not have led one of ordinary skill in the art to the claimed methods with any reasonable expectation of success. The Raviprakash *et al.* publication discloses that unmodified antisense oligonucleotides were not effective in bringing about significant inhibition of DV (see abstract), and that a modified antisense oligonucleotide targeting a region in the DV 3' untranslated region (UTR) (the 3'b-AS oligonucleotide) was less effective than a modified antisense oligonucleotide targeting a region near the DV translation initiation site (the 5'-AS oligonucleotide). Raviprakash *et al.* attributed this result to the complex secondary structures presented by the large (>10 kb) DV RNA (page 74, first full paragraph). In contrast to these results with 3' UTR-targeted antisense oligonucleotides, Applicants note that the subject specification demonstrates very effective inhibition of DV infection and DV-induced apoptosis in human dendritic cells. Finally, the Raviprakash *et al.* publication concludes that the modified phosphorothioate oligonucleotides may be generally more effective as antisense agents against other viruses (page 74, last sentence). In view of the difference in effectiveness between antisense oligonucleotides targeting the DV 3' UTR and antisense oligonucleotides targeting a region near the translation initiation site observed by Raviprakash *et al.*, and in view of the recognition in the art that the accessibility of target sequences within regions having secondary

structures affects the effectiveness of siRNA, as it does antisense oligonucleotides, one of ordinary skill in the art would not reasonably conclude that targeting the DV 3' UTR and switching from an antisense approach to an siRNA approach would be effective.

Applicants respectfully submit that, at the time the subject application was filed, even successful targeting of specific regions with antisense oligonucleotides did not necessarily confer a reasonable expectation of success in those regions with interfering RNA. Again, in Raviprakash *et al.*, the unmodified oligonucleotides were not effective. Accessibility of target regions by antisense oligonucleotides did not necessarily confer a reasonable expectation of success by the effector complex (the RNA-induced silencing complex (RISC)) in RNA interference.

The Adelman *et al.* publication describes intrathoracic injection of mosquitos (*Aedes aegypti*) with vectors encoding sense and anti-sense RNA-mediated interference molecules. While the Adelman *et al.* publication suggests new ways of inhibiting replication of DV in mosquito vectors, it is not relevant to inhibition of dengue virus replication in human cells or a human.

The Yu *et al.* publication is cited in the Office Action for teaching RNA interference by expression of hairpin siRNAs and their use in mammalian cells. The Office Action concludes that one skilled in the art would have been motivated to substitute the antisense oligonucleotides of the Iversen *et al.* publication because the Tuschl *et al.* publication taught that siRNAs were more efficient than antisense. The Yu *et al.* and Tuschl *et al.* publications do not address the aforementioned deficiencies of the Iversen *et al.*, Raviprakash *et al.*, and Adelman *et al.* references.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art. MPEP §2143.01. Obviousness does not require absolute predictability, however, at least some degree of predictability is required. MPEP §2143.02. Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. *In re Rinehart*, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976). Furthermore, assuming *arguendo* that it would have been obvious to try administering the 3' NCR-targeted vector to human cells *in vivo* to attenuate DV infection, it is well established that obvious to try is an acceptable rationale in support of a conclusion of obviousness when choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success. MPEP §2141. Such is not the case here, for the reasons cited

above.

Furthermore, as indicated by Subramanya *et al.*, dengue pathogenesis is characterized by overproduction of proinflammatory cytokines, including TNF-alpha, which is implicated in the vascular leakage that characterizes DHF/DSS and the plasma levels of which are elevated during acute dengue infection (see page 2498, right column, of Subramanya *et al.*). Therefore, an important limitation to be considered is whether blockade of host molecules such as TNF-alpha "also interferes with a possible antiviral effect that might outweigh its pathogenic potential." Not only did the inventors of the subject invention empirically determine that interfering RNA could be successfully delivered to human dendritic cells, decrease DV infection, and inhibit DV-induced apoptosis of human dendritic cells, the inventors determined that the interfering RNA did not induce acute inflammation in human dendritic cells as determined by the level of pro-inflammatory cytokines, including TNF-alpha, as shown in Figure 10 and described in Example 10 at page 31 of the subject specification. It is noted that, at page 13, the Office Action indicates that Applicants

> appear to be arguing limitations that are not in the claims. It is noted that claim 74 requires that the adenoviral vector does not induce inflammation in dendritic cells, but this is considered to be an inherent property of adenoviral vectors. The claims do not recite any limitations regarding blockade of TNF alpha.

In response, Applicants respectfully submit that in determining the differences between the cited references and the claims, the claimed invention as a whole must be considered. MPEP §2141.02 makes clear that "in determining whether the invention as a whole would have been obvious under 35 U.S.C. §103, we must first delineate the invention as a whole. In delineating the invention as a whole, we look not only to the subject matter which is literally recited in the claim in question... but also to those properties of the subject matter which are inherent in the subject matter *and* are disclosed in the specification. . . Just as we look to a chemical and its properties when we examine the obviousness of a composition of matter claim, it is this invention *as a whole,* and not some part of it, which must be obvious under 35 U.S.C. §103." *In re Antonie*, 559 F.2d 618, 620, 195 USPQ 6,8 (CCPA 1977). In fact, evidence and arguments directed to advantages not disclosed in the specification cannot be disregarded. "The totality of the record must be considered when

determining whether a claimed invention would have been obvious to one of ordinary skill in the art at the time the invention was made." MPEP §716.02(f).

The Yu *et al.* patent is relied upon in the Office Action for teaching a variety of methods to deliver nucleic acids to cells. The Yu *et al.* patent does not cure those deficiencies or confer a reasonable expectation of success in administering the 3' NCR-targeted vector to human cells susceptible to DV infection *in vivo* or human dendritic cells *in vivo*, as recited in the currently pending claims.

The Hope *et al.* patent is relied upon in the Office Action for teaching that expression cassettes could be delivered by a variety of viral or non-viral vectors, including adeno-associated virus. The deficiencies of the other references are described above. The Hope *et al.* patent does not cure those deficiencies or confer a reasonable expectation of success in administering the claimed vector to human cells susceptible to DV infection *in vivo* or human dendritic cells *in vivo*.

Applicants respectfully submit that the claimed methods are not obvious over the cited references as there would have been no motivation to select siRNA target sequences falling outside of the large open reading frame encoding the DV polyprotein and within the 3' non-coding region, with any reasonable expectation of success in achieving inhibition of DV infection *in vivo*. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

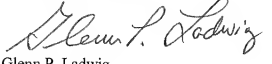
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



Glenn P. Ladwig
Patent Attorney
Registration No. 46,853
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: Saliwanchik, Lloyd & Eisenschenk
A Professional Association
P.O. Box 142950
Gainesville, FL 32614-2950

GPL/jnw
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